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SARAWUT SUTTIRUT : COMPARISON OF ELISA AND DOT BLOT HYBRIDIZATION WITH AGAROSE GEL ELECTROPHORESIS FOR RAPID DETECTION OF PCR PRODUCTS FROM *MYCOBACTERIUM TUBERCULOSIS*. THESIS ADVISORS : UNCHALEE TANSUPHASIRI, MS., MR. SOMSAK RIENTHONG, M.Sc. 157 P. ISBN 974-662-983-2

A microplate ELISA hybridization assay which uses IS*6110* as the target for amplification was developed for the rapid detection of the PCR products from *Mycobacterium tuberculosis* from clinical cultures and clinical samples. This assay was then evaluated in comparison to two other detection methods: Agarose Gel Electrophoresis (AGE), and Dot Blot Hybridization (DBH). The sensitivity, specificity, positive predictive value (PPV), negative predictive value (NPV), and accuracy were calculated in comparison with culture results which used as the "gold standard" for diagnosing *M. tuberculosis* infection. The assay is based on the specific detection with fluorescein-labeled detection probe of biotinylated PCR products which are captured in avidin coated microplate. Hybridized products with fluorescein were identified by using anti-fluorescein antibody horse radish peroxidase conjugate and colorimetric peroxidase substrate. This assay discriminated perfectly between the positive and negative groups, when an OD at 490 nm of 0.18 was used as the cut off point.

The agreement rates of PCR product detection from 56 clinical cultures by AGE comparing with DBH and ELISA were 0.964 and 0.964, respectively, while that of DBH and ELISA was 1.0 by Kappa analysis. The overall agreement was not statistically significantly different ($P>0.05$). The assay was used with 190 sputum samples and the PCR results between ELISA and AGE were highly agreeable ($K = 1.0$ by Kappa analysis) with sensitivity, specificity and accuracy of 89.9, 100, 95.8%, respectively. The same values for DBH were 92.4, 98.2 95.8 %, respectively. In comparison, the validities of these three PCR detection methods were not statistically significant different ($P>0.05$). Use of DBH or ELISA hybridization increased the sensitivity of detection by PCR-AGE assay by 10-fold from 10 pg to 1 pg of purified DNA per reaction; i.e., from about 30 to about 3 *M. tuberculosis* H37Rv organisms. The amount of PCR product that could be detected by ELISA was only one half that detected by the other methods; the total assay time of ELISA following the PCR was 4 hours.

In conclusion, the ELISA hybridization assay can replace conventional AGE and DBH for the rapid detection of the PCR products from *M. tuberculosis* because of its sensitivity, specificity and accuracy were not different from those methods. ELISA has several advantages over AGE and DBH in that it is less cumbersome, more rapid, less hazardous, cost effective, results may be read objectively, and suitable for use in epidemiological studies for the analysis of large numbers of samples.